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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 3 :
A61K 37/02, 37/04, 35/16
C07G 7/00

A1

(11) International Publication Number: WO 82/ 04395

(43) International Publication Date: 23 December 1982 (23.12.82)

(21) International Application Number: PCT/SE81/00183

(22) International Filing Date: 18 June 1981 (18.06.81)

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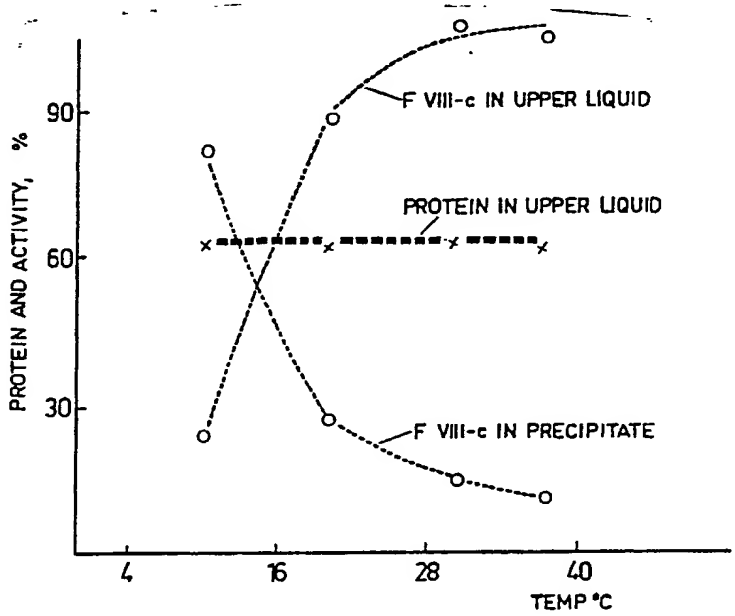
(81) Designated States: AT (European patent), BR, CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), JP, NL (European patent), NO, SE (European patent), SU, US.

Published
With international search report.

(54) Title: A PROCESS IN PURIFICATION AND CONCENTRATION OF THE FACTOR VIII COMPLEX



(57) Abstract

A process in purification and/or concentration of factor VIII complex, starting from a preparation of factor VIII complex, obtained and concentrated in a known manner, mostly in the form of a precipitate, such as cryoprecipitate or Cohn's fraction I-0. This preparation is dissolved in a glycine solution of at least 1.5 M at a temperature of at least +15°C and pH of 6.3-7.8 and a supernatant liquid is recovered as a product or for further working up, particularly precipitation of the factor VIII complex from the supernatant liquid with more than 1.5 M salt solution maintaining the glycine concentration of at least 1.5 M.

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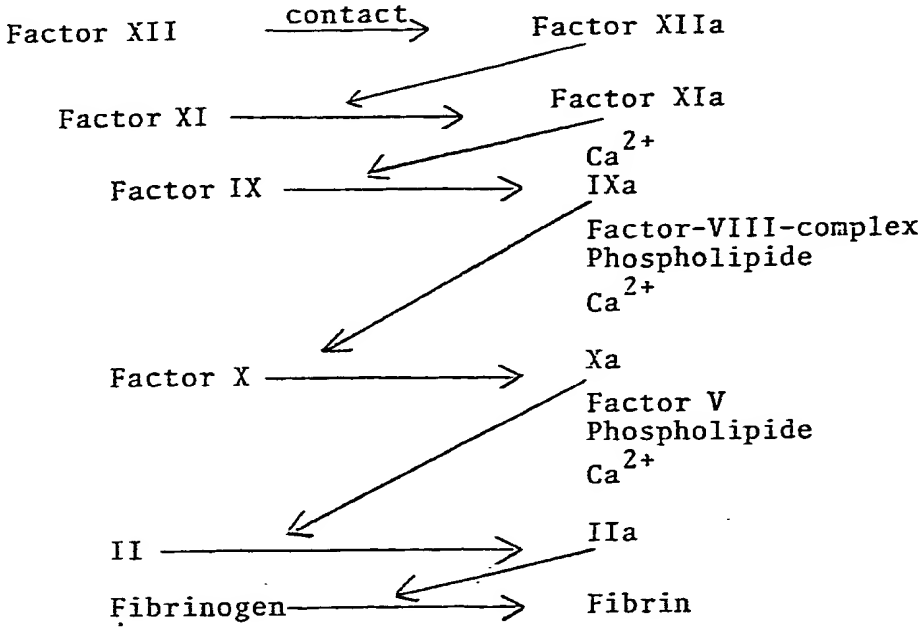
A process in purification and concentration of the factor VIII-complex

This invention relates to a process in purification and/or concentration of the factor VIII complex. It is started from a preparation prepared in a manner known per se, in which the factor VIII complex is enriched. The preparation is mostly in the form of a precipitate.

The factor VIII complex is lacking or its activity is reduced in hemophilia of type A and in v. Willebrand's disease. The symptoms in these diseases are often serious bleedings in joints and muscles and from mucous membranes.

The factor VIII complex takes part in the biochemical reactions promoting the coagulation of blood. In the coagulation of blood, an insoluble polymer, fibrin, is formed from a soluble protein, fibrinogen. The reason for the polymerization or formation of fibrin is an enzymatic change of the fibrinogen molecule, which is caused by the enzyme trombin (factor IIa). This enzyme is formed from protrombin (factor II) under the influence of an enzyme, called factor Xa. Said enzyme is also present as a zymogen in the blood before the coagulation. The zymogen form is called factor X. The conversion of factor X to factor Xa also takes place enzymatically by means of an enzyme called factor IXa. The factor VIII complex takes part in this reaction (as a co-factor) together with calcium and phospholipide. In the following scheme the most important reactions in the coagulation (clotting) of blood are illustrated. Totally a great number of various factors take part here.



The factor VIII complex consists of at least two components, one of which is called the factor VIII:C, in which C means that this component is responsible for the coagulation activity of the complex in the reaction chain shown. This component is considered to contain the antigen proved by means of antibodies, which are developed in certain persons suffering from hemophilia and which prevent the coagulation activity of factor VIII:C. The antigen is called F VIII:CAG. The other component has been called factor VIII-RAG or F VIII-related antigen. This antigen is different from the antigen F VIII:CAG. The factor VIII:C and the (antigen) factor VIII:CAG are lacking in hemophilia of type A in a serious form. In this disease there is a normal content of factor VIII:RAG. In v. Willebrand's disease, there is a lack of factor VIII:RAG in the blood and a corresponding lack of factor VIII:C. For persons suffering from a serious form of v. Willebrand's disease the lack of factor VIII:RAG is almost total and the content of factor VIII:C is about 5 % of the normal content. In v. Willebrand's disease, the activity of the so-called factor VIII:RCF is highly reduced. This activity is an expression of a component in

plasma which causes agglutination of trombocytes in the presence of the antibiotic "Ristocetin". It is now considered that the activity of the factor VIII:RCF is an expression of the component or components in plasma which has (have) the factor VIII-related antigen (factor VIII: RAG). The lack of factor VIII:RCF in v. Willebrand's disease has been found to be correlated to the prolonged capillary bleeding time existing in this disease and which is an expression of a defective trombocyte function. This function is normal in hemophilia type A, whereas the coagulation time of the blood is prolonged due to the reduced content of factor VIII:C. In v. Willebrand's disease, the coagulation time is also prolonged as there is also a lack of factor VIII:C in this disease, especially in serious forms thereof. The lack of factor VIII:C in v. Willebrand's disease is considered to be a consequence of the lack of factor VIII:RAG/RCF, which seems to act as carrier molecule for factor VIII:C. In accordance with the existing values, one can illustrate the factor VIII--complex schematically in the following way:

F VIII:RAG/RCF = = F VIII:C/CAG

A number of processes are known at present for the preparation of plasma concentrates for clinical use, which contain the factor VIII complex or parts thereof. The complex can be precipitated from Cohn's fraction I with alcohol. Additional concentration of the complex can be carried out by extraction of inert protein whith about 1 M glycine solution in cold. Fractionation of plasma with ether or tannic acid has also been used for the preparation of factor VIII concentrates. Concentrates prepared by precipitation in cold of the factor VIII complex have been widely used due to the simplicity of the method. In certain cases, polyethylene glycol has been used for precipitation of the factor VIII complex. Variants of the glycine method, in which either other amino acids than glycine have been used or glycine has been used for precipitation of the factor VIII complex are also known.

Concentrates prepared according to the glycine method contain all factors in the factor VIII complex, but the specific activity is low, and therefore great volumes of solution must be injected in the treatment of hemophilia. Preparations with a high specific activity have often been found to lack the factor correcting the prolonged bleeding time in v. Willebrand's disease.

As to the yield of the coagulation active part (F VII:C), this is low in most preparation processes or between 20 and 30 % at best.

In the treatment of hemophilia, it is of a great importance to use concentrates of the F VIII complex with a high specific acitivity of all the factors included in the complex. Hemophilia A as well as v. Willebrand's disease can be treated with such a concentrate. A specific activity which is 200-300 times greater than that in plasma also permits preparations containing the necessary therapeutic dose in a small volume (5-10 ml). This makes the treatment easier as the dose can be administered in an injection syringe. This also makes it possible to carry out the treatment for example at home by the sick person himself as is usual in the treatment of diabetes with insulin.

In most countries, the supply of blood is unsatisfactory in respect of the possibilities of preparing sufficient amounts of factor VIII complex for treatment of hemophilia from available blood volumes. This situation might be considerably improved if the percentage yield of the factor VIII complex could be increased in preparation from blood.

It has now been found to be possible to prepare a highly satisfactory factor VIII complex having a specific activity that is about 150-250 times greater than that in plasma, while the yield is between 40 and 60 %. The process according to the invention is charactericed in that the resulting preparation of factor VIII complex is dissolved in a glycine solution of at least 1.5 M at a temperature of at least +15°C and a pH of 6.3-7.8, preferably pH 6.8, and that supernatant liquid is recovered as a product or for

further working-up. The resulting product (factor VIII complex) contains the antihemophilic factor (factor VIII:C/CAG) and the component (factor VIII-RAG/RCF) which is lacking in v. Willebrand's disease.

The starting preparation obtained in a way known per se can be prepared by cryoprecipitation or fractionation of plasma with alcohol. Through the process of the invention, impurities are then precipitated in a glycine solution at and about room temperature and at a neutral pH. In previously known processes, glycine solutions have been used at a low temperature for dissolution of impurities from a precipitated factor VIII complex. Glycine at a concentration of about 2 M has also been used for precipitation of the factor VIII complex from a protein solution at a low temperature.

According to the invention, the preparation of factor VIII complex is now treated at a temperature of at least 15°C, preferably at a temperature of 15-37°C, such as 20-35°C, and most preferably at about 30°C. The temperature should not normally exceed 40-45°C.

In doing this, the factor VIII complex remains in solution, while contaminating protein is precipitated. The pH is approximately neutral. The ionic strength of the solution should be between 0.1 and 0.5, calculated as buffer salts. The glycine concentration at the precipitation of impurities can vary between 1.5 M and a saturated solution and is preferably 1.6-2.5 M, such as 1.8-2.2 M, and most preferably about 2.0 M. By this precipitation of protein which is inert in this connection, the specific activity in the supernatant liquid is increased by 2-7 times and the yield is between 80 and 100 %.

The supernatant liquid can be worked up in a manner known per se by precipitating the factor VIII complex with organic solvent or polymerisolutions. However, according to a novel technic this factor VIII complex also can be precipitated by the addition of salt. Thus, salt is added to the supernatant liquid to a concentration of at least

1.5 M maintaining the glycine concentration of at least 1.5 M to precipitate the factor VIII complex containing product which is recovered. The salt used should be non-toxic and should be able to provide the necessary concentration of at least 1.5 M, e.g. 1.8 M, preferably about 2.0 M or more, e.g. 2.2 M. It is possible to use a saturated salt solution. The glycine concentration can be the same as in the dissolving step. The temperature can be as low as the freezing point of the supernatant liquid up to as high temperatures as e.g. 50°C or 60°C. Temperatures below 40°C are preferred, as 0-37°C, e.g. 2-30°C. Examples of salts that can be used are NaCl, KCl, $CaCl_2$, $MgCl_2$, NH_4Cl, $(NH_4)_2SO_4$, Na_2SO_4, K_2SO_4, Na_3PO_4, Na_2HPO_4, NaH_2PO_4 and citrates of sodium, potassium and ammonium; thus alkaline metal salts, such as sodium and potassium salts and ammonium salts, are preferred.

The invention is described more in detail in the following examples with reference to its use in the fractionation of blood plasma. In the examples, it is referred to the enclosed drawing, in which Fig. 1 shows protein and activity in the supernatant liquid and the specific activity as a function of the molarity of the glycine solution. Fig. 2 shows the activity in supernatant liquid and in precipitate and protein in the supernatant liquid as a function of the temperature. In Figs. 3 and 4 the same quantities are shown as a function of pH and ionic strength, respectively. Fig. 5 shows the activity in vivo of the factor VIII concentrate in a patient suffering from severe v. Willebrand's disease, and also the inverted value of the bleeding time; Fig. 6 is a diagram illustrating the yield of factor VIII in the supernatant liquid and the precipitate versus the salt concentration.

Example 1. Blood is collected in citrate-phosphate-dextrose-adenine solution in an amount of 50 ml of citrate solution per 450 ml of blood. Blood cells are removed by centrifuging and plasma is sucked off. The plasma is fractionated with alcohol according to Cohn's fractionation method. Fraction I is washed in cold with glycine solution.

The remaining precipitate, called fraction I-0, contains factor VIII complex and is dissolved in sodium citrate solution (pH 6.8) of 0.055 M to a protein concentration of about 2 %. 8 parts of a buffer with a pH of 6.8 and containing varying amounts of glycine, 0.125 M NaCl and 0.025 M imidazole are added to 4 parts of this solution at room temperature. After stirring for 15 minutes the precipitate formed is removed by centrifugation at ambient temperature. The precipitate is dissolved in citrate solution. The precipitate as well as the supernatant liquid is analyzed in respect of activity of factor VIII:C (normalization of the coagulation time of plasma from persons with hemophilia A after recalcification) or with a commercial reagent (IMCO AB, Stockholm). The total protein is determined by means of Lowry's method. The results of this test appear from Fig. 1. As shown in the figure, considerable amounts of inert protein are precipitated from the solution of fraction I-0 at glycine concentrations of 1.1-2.2 M. When the glycine content increases over 2.2, additional precipitation of inert protein takes place, but considerable losses of factor VIII:C can occur from the supernatant liquid. At a glycine content of 1.5-2.0 M, the yield of factor VIII:C in the supernatant liquid is high, while the content of protein therein is much lower than in the original solution. The specific activity of the supernatant liquid is about 5 times higher than in the original solution at a glycine concentration of 2 M.

Example 2. Blood and plasma are prepared in the way described in example 1. A counterpart of fraction I-0 is prepared by freezing of the factor VIII complex by adding polyethylene glycol 4000 in an amount of 1 % to the plasma. The mixture is frozen at -70°C. At a slow thawing to +4°C a precipitate called cryoprecipitate remains when all ice has melted. This precipitate contains between 60 and 90 % of the factor VIII complex of the plasma. The precipitate is dissolved in 0.055 M sodium citrate solution (pH 6.8) to a protein content of about 3 %. 10 parts of a buffer

containing 3.0 M glycine, 0.125 M NaCl and 0.025 M imidazole (pH 6.8) are added to 5 parts of this solution at 10°, 20°, 30° and 37°C. The mixtures are brought to equilibrium at the different temperatures under stirring for 15 min. The precipitate formed is removed by centrifugation. Precipitate and supernatant liquid are analyzed as in example 1.

As is apparent from Fig. 2, the separation of the factor VIII complex from inert protein is highly dependent on the temperature of the glycine-protein mixture. At temperatures above 15°C, the main part of the factor VIII complex is found in the supernatant liquid, while at lower temperatures, the main part is in the precipitate.

Example 3. The cryoprecipitate (see example 2) is dissolved in a 0.055 M citrate solution (pH 6.8) to a protein content of about 3 %. 8 parts of buffer containing 3 M glycine, 0.125 M NaCl and 0.025 M imidazole, the pH of which has been adjusted to 6.3; 6.8; 7.3 and 7.8, respectively, are added dropwise to 4 parts of this solution. After stirring for 15 min at 30°C, the precipitate formed is removed by centrifugation and dissolved in citrate solution (pH 6.8). The supernatant liquid and the precipitate are analyzed according to example 1.

As is evident from Fig. 3, the differences in activity and yield at different pH are relatively small. An optimal separation of the factor VIII complex on behalf of supernatant liquid is obtained at approximately neutral pH.

Example 4. 10 parts of glycine-imidazole solution with a varying content of common salt were added dropwise to 5 parts of the cryoprecipitate (see examples 2 and 3). The composition of the different additional solutions is as follows:

1) 3 M glycine, 0.025 M imidazole, pH 6,8; 2) 3 M glycine, 0.025 M imidazole, 0.3 M NaCl, pH 6.8; 3) 3 M glycine, 0.025 M imidazole, 0.6 M NaCl, pH 6.8.

After achieving equilibrium at 30°C for 15 min the resulting precipitates are removed by centrifuging. The

supernatant liquid and the precipitate are analyzed according to example 1.

The results are shown in Fig. 4. The variation in ionic strength between 0.1 and 0.5 has only a small effect on the distribution of the factor VIII complex in supernatant liquid and precipitate.

Example 5. Fraction I-0 is dissolved in citrate solution (see example 1) to a protein content of about 2 %. 10 parts of 3 M glycine containing 0.125 M NaCl and 0.025 M imidazole, pH 6.8, are added to 5 parts of the resulting solution. After stirring at 20°C the precipitate (F I) is removed by centrifugation. The supernatant liquid (upper liquid I) is cooled to 4°C. Aqueous polyethylene glycol 6000 of 30 % is then added to a final concentration of 10 %. After stirring for 30 min at 4°C, the precipitate (F II) is removed from supernatant liquid (upper liquid II) by centrifuging. All supernatant liquids and precipitates are analyzed in the way described in example 1.

The distribution of the protein and the factor VIII complex in the different fractions is apparent from the following table 1. As is apparent from the table, the fraction I-0 is suitable as a starting material for fractionation. The results show, which is most important, that the factor VIII complex in the supernatant liquid can be concentrated by precipitation with polyethylene glycol after precipitation of inert protein with glycine.

Table 1

Fraction	Vol. mls	Protein mg/ml	Total protein mg	%	VIII:C E/ml	Total E	VIII:C %	Specific activity E/mg
F I-0	18	19.8	357	100	2.36	42.5	100	0.12
Upper liquid I	50	-	-	-	0.66	33.0	77.6	-
F I	19	11.5	219	61.3	0.49	9.3	21.6	0.04
Upper liquid II	73	-	-	-	0.14	10.2	24.0	-
FII	3.7	16.6	61	17.1	5.94	22.0	51.8	0.36

Example 6. 10 parts of solution containing 3 M glycine, 0.125 M NaCl and 0.025 M imidazole at pH 6.8 are added to 5 parts of solution of the cryoprecipitate (see example 4) at 20°C. After stirring for 10 min the precipitate (F I) formed is removed by centrifuging. The supernatant liquid (Upper liquid I) is cooled to 4°C, after which an aqueous solution of 50 % of polyethylene glycol 6000 is added dropwise to a concentration of 6 %. After stirring for 30 min the precipitate (F II) is removed from the supernatant liquid (upper liquid II)by centrifugation. All fractions are analyzed in the way described.

The results of the test appear from table 2. It is apparent from this that cryoprecipitate as well as fraction I-0 is suitable for this type of fractionation. The test also shows that the factor VIII complex, which according to the invention has been obtained in the supernatant liquid, can be concentrated by precipitation with polyethylene glycol.

Table 2

Fraction	Vol. mls	Protein mg/ml	Total protein mg	%	VIII:C E/ml	Total E	VIII:C %	Specific activity E/mg
Cryo	18.5	26.4	488	100	5.57	103.0	100	0.21
Upper liquid I	53	-	-	-	1.72	91.2	88.5	-
F I	22.5	9.6	216	44.3	0.63	14.2	13.8	0.07
Upper liquid II	31	-	-	-	0.09	2x 2.8	5.4	-
F II (H)	8.8	3.2	2x 28.2	11.6	3.77	2x 3.2	64.5	1.18

Example 7. Cryoprecipitate (see example 2) is dissolved in 0.055 M citrate solution, pH 6.8, to a concentration of 3 %. 3.3 parts of a solution containing 2.6 M glycine and 0,025 M imidazole, pH 7.3, are added to 1 part of this solution. The temperature of the mixture is simultaneously brought to 7°C. After the addition, the mixture is brought to equilibrium under stirring at 7°C for 15 min. A precipitate (F I) is formed and removed by centrifugation. The supernatant liquid (Upper liquid I) is saved for analysis. The precipitate is dissolved in 0.055 M citrate solution, pH 6.8, at 30°C to about the same volume as the original cryoprecipitate, after which 3.3 volumes of a solution containing 2.6 M glycine, 0.3 M NaCl and 0.025 M imidazole, pH 6.8, are added. Stirring is carried out at 30°C for 20 min. The precipitate (F II) formed is removed by centrifugation. The supernatant liquid (Upper liquid II) is adjusted to pH 7.5 with a weak sodium hydroxide solution after cooling to 4°C. An aqueous solution of 30 % polyethylene glycol 6000 is then added dropwise to a concentration of 6.5 %. The mixture is stirred at 4°C for 30 min, after which the precipitate formed is removed by centrifuging. The precipitate (F III) is dissolved in 0.055 M sodium citrate solution containing 0.055 M imidazole, pH 7.40. The supernatant liquid (Upper liquid II) is saved for analysis. All fractions are analyzed in the way described in example 1, and the results appear from the following table 3.

Table 3

Fraction	Vol. mls	Protein mg/ml	Total protein mg	%	VIII:C E/ml	Total E	VIII:C %	Specific activity E/mg
Cryo	56	30.4	1702	100	4.29	240	100	0.14
Upper liquid I	228	-	-	-	0.25	57	23.8	-
F I	40	21.1	844	49.6	5.96	238	99.2	0.28
Upper liquid II	168	-	-	-	0.97	163	67.9	-
F II	30	23.5	705	41.4	0.49	15	6.3	0.02
Upper liquid III	198	-	-	-	0.04	8	3.3	-
F III	6.2	13.4	83	4.9	27.8	172	71.7	2.07

Example 8. In the previous examples, the analyses of the factor VIII complex have only comprised factor VIII:C. In the following table 4 analyses of a factor VIII complex is shown, which has been isolated substantially in the way described in example 7.

Table 4

Fraction	Prot.	VIII:C	VIII:CAG	F VIII:RAG	VIII:RCF	Spec. activity
Cryo	100	100	100	100	100	0.15
F III	4	63	61	79	48	2.1

Remark: The factor VIII:C can be determined according to Nilsson et al: Acta Med. Scan. 159, 35 (1953) or according to Savidge et al: Thromb. Res. 16, 355 (1979). The factor VIII:CAG can be determined according to Lazarchick and Hoyer, J. Clin. Invest. 62, 1048 (1978).

It is apparent from the table that the complex, expressed in percent, contains the same concentration of coagulation factor VIII:C as of factor VIII:CAG. The content of factor VIII:RAG is somewhat higher and the content of factor VIII:RCF (lacking in v. Willebrand's disease) is somewhat lower than of factor VIII:C/CAG.

It is evident that products with a high specific activity and a high solubility can be obtained in a good yield at fractionation with glycine according to the invention in combination with other frationating processes. Furthermore, these preparations contain all the activities which in addition to F VIII:C are associated with the factor VIII complex. In comparison with plasma (the specific VIII:C activity of which is about 0.014 U/mg), the specific activity of the product will be 150-250 times greater, the yield from plasma under optimal conditions being 40-60 %.

Example 9 The cryoprecipitate is prepared as in example 2 and dissolved in 0.555 M sodium citrate solution, pH 7.30, to a protein content of about 3%. A solution consisting of 2.6 M glycine, 0.3 M NaCl and 25 mM tris is thereafter added at a temperature of 20-30°C to a final concentration of 2 M glycine. Stirring is carried out for 20 min. The precipitate formed is separated by centrifugation. The supernatant is cooled to 4°C, after which solid sodium chloride is added to a final concentration of 2 M. Stirring is carried out for 30 min. after which the precipitate formed is separated by centrifugation. This precipitate is dissolved in 0,055 M sodium citrate solution at a pH of 7.30. The procedure described above gives for F VIII:C a final product in a yield and purification of 30-40% and 195 times, respectively, calculated from plasma and for F VIII:RCF 60-70% and 430 times, respectively. In a clinical test on a patient suffering from a serious form of v. Willebrand's disease this concentrate has normalized the prolonged bleeding time and increased the level of F VIII:C in plasma to 55% (Fig. 5). This shows that the preparation is suitable in treatment of patients with hemophilic diseases.

The inverted value ($\frac{1}{\text{min}}$) of the bleeding time is also plotted versus the period of time (hours) elapsed after injection to this patient. The Iwy's method [Nilsson, Magnusson, Borchgrevink, Thr. Diat. Hamorrh, 10, 223 (1963)] and the Duke's method [J.A.M.A. 55:1185-1192 (1910)] have been used. Normal inverted bleeding time is more than 0.2 min^{-1} and 0.43 min^{-1} for Iwy's method and Duke's method respectively.

Example 10. A supernatant liquid is prepared from a cryoprecipitate as in example 9. This supernatant is cooled to 4°C and divided in three aliquots; thereafter, solid sodium chlorid is added to each aliquot to a final concentration of 1.0, 1.5 and 2.0 M, respectively. Each aliquot is then treated in the following way: stirring is carried out for 30 minutes, after which the precipitation formed is separated by centrifugation. The precipitation is dissolved in 0.055 M sodium citrate solution at a pH of 7.30. The yield of factor VIII is plotted versus the concentration of NaCl in the diagram of fig.6. The line O II and the line F II show the yield, based on the cryoprecipitate, of factor VIII in the supernatant liquid and in the precipitation, respectively.

Example 11. Example 9 is repeated with the exception that room temperature is used instead of 4°C during the precipitation with sodium chloride. The following table gives the result from this example; thus the volume and the content of the factor VIII:C is given in units as well as percentage in the cryoprecipitate, the supernatant liquid from the solution process with 2 M glycine solution and in the salt precipitate from the precipitation with 2 M sodium chloride.

Fraction	Vol mls	Factor VIII:C U	%
Cryoprecipitate	100	463	100
Supernatant	395	506	109
Salt precipitate	13,5	304	66

CLAIMS

1. A process in purification and/or concentration of factor VIII complex, starting from a preparation of factor VIII complex obtained and concentrated in a known manner, most often in the form of a precipitate, c h a r a c t e r i z e d in that the preparation is dissolved in a glycine solution of at least 1.5 M at a temperature of at least +15°C and a pH of 6.3-7.8, and that a supernatant liquid is recovered as the product or for further working up or that optionally salt is added to the supernatant liquid to a concentration of at least 1.5 M maintaining the glycine concentration of at least 1.5 M to precipitate the factor VIII complex containing product which is recovered.

2. The process of claim 1. c h a r a c t e r i z e d in that it is started from a cryoprecipitate or from Cohn's fraction I-0.

3. The process of claim 1 or 2. c h a r a c t e r - i z e d in that the ionic strength of the solution is 0.1-0.5.

4. The process of any of claims 1-3, c h a r a c - t e r i z e d in that the temperature is between 20 and 40°C, preferably 25-37°C.

5. The process of any one of claims 1-4, c h a r a c- t e r i z e d in that pH is adjusted to 6.5-7.5, preferably to about 6.8, before the precipitation with glycine solution.

6. The process of any one of claims 1-5, c h a r a c- t e r i z e d in that the glycine solution is a saturated solution, preferably a solution of 1.8-2.2 M, such as about 2.0 M.

7. The process of any one of claims 1-6, wherein the salt is sodium chloride.

8. The process of any one of claims 1-7, wherein the salt precipitation is carried out at a temperature from the freezing point of the supernatant to 60°C.

FIG.1

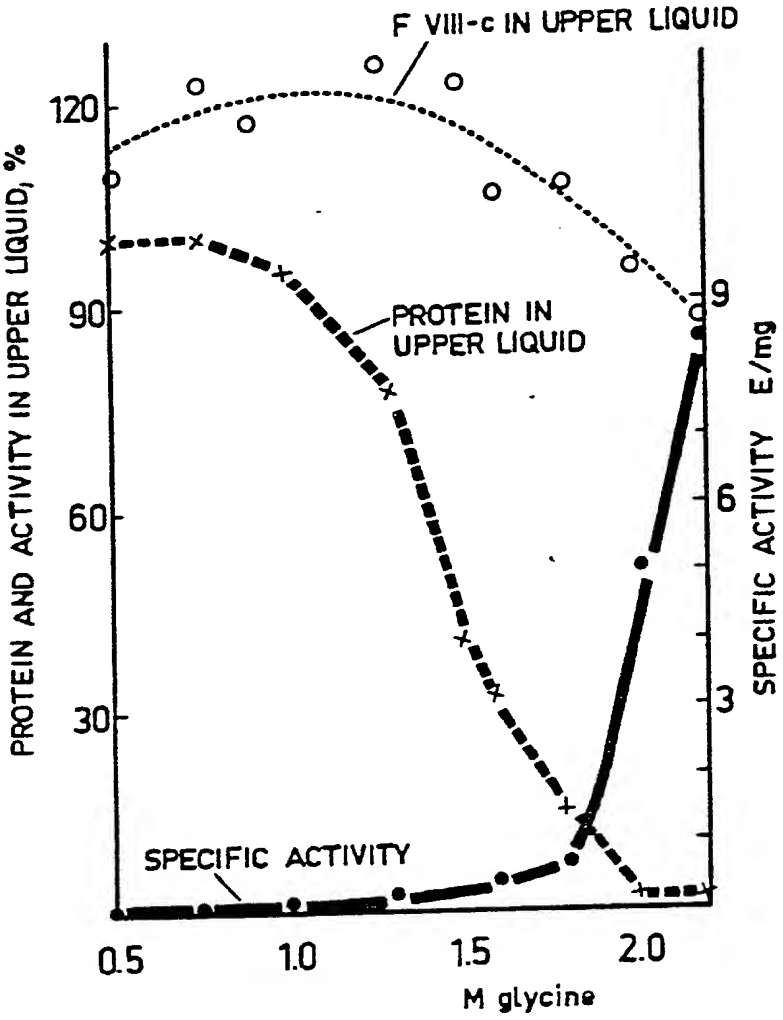


FIG. 2

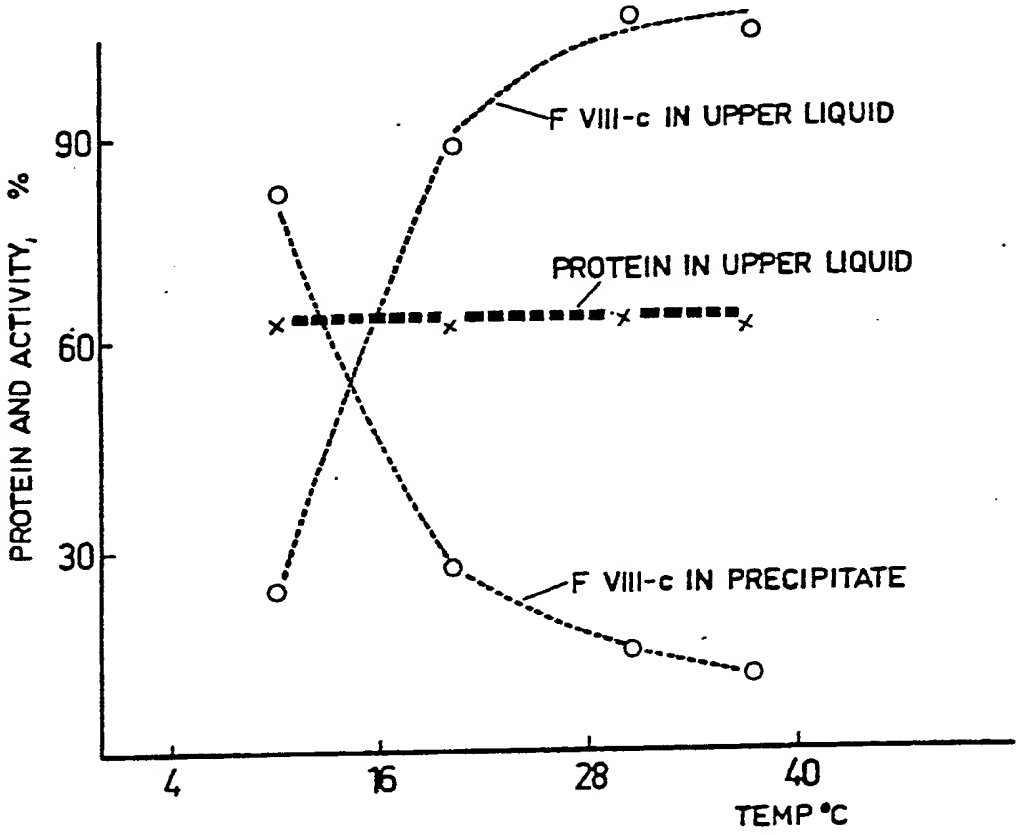


FIG. 3

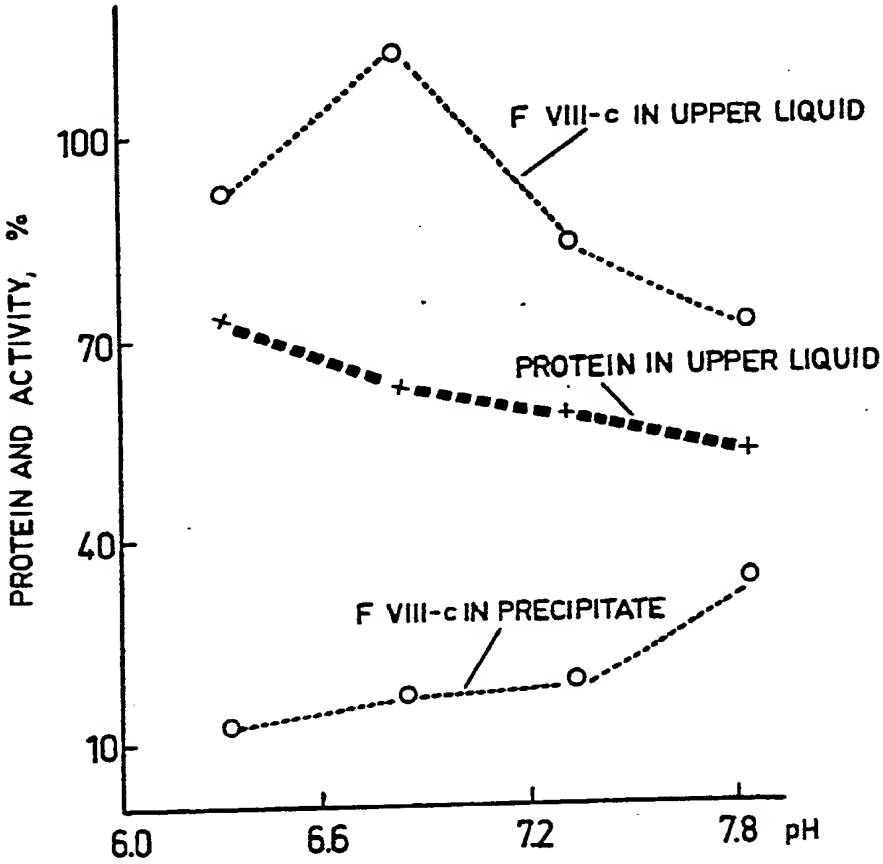


FIG.4

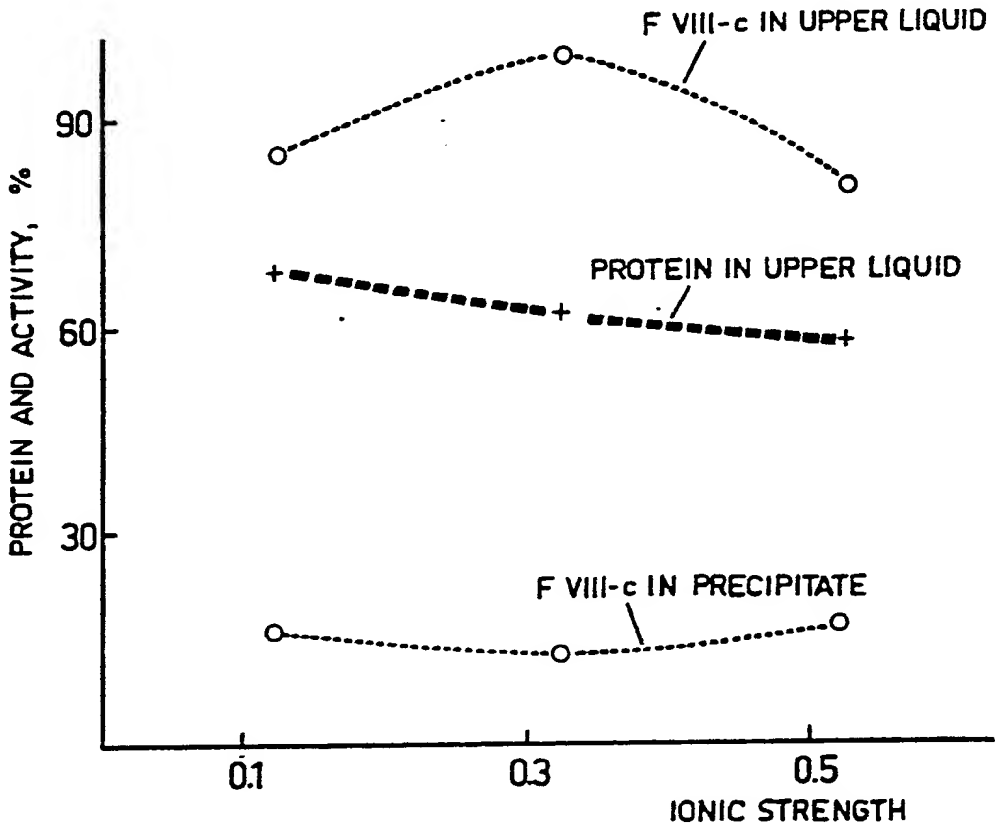


FIG.5

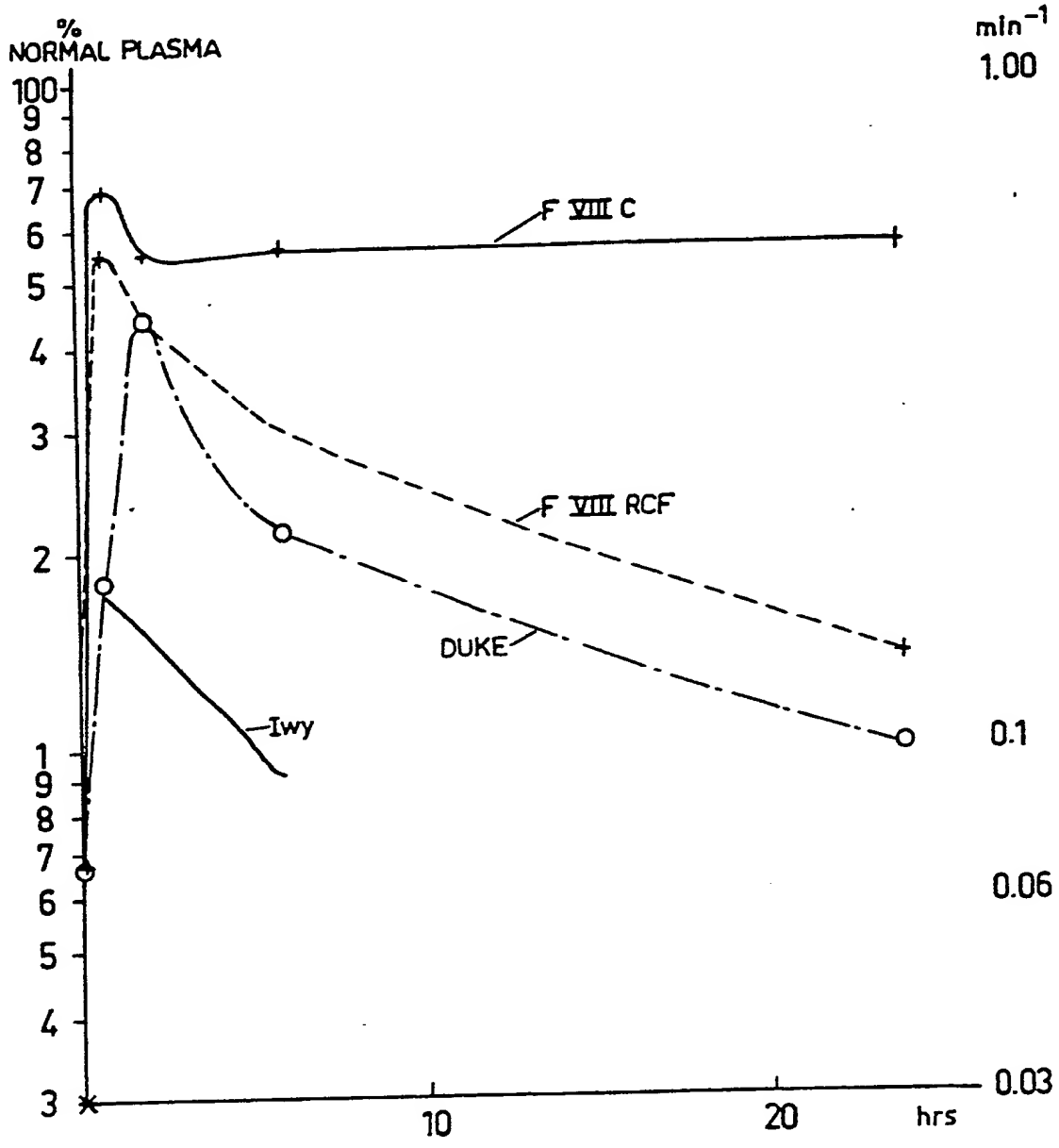
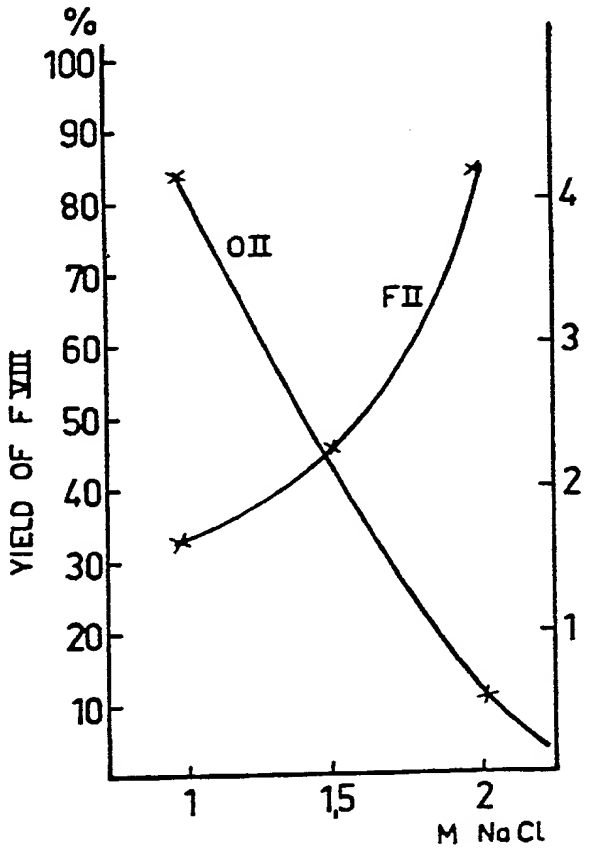


FIG.6



INTERNATIONAL SEARCH REPORT

International Application No PCT/SE81/00183

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC 3

A 61 K 37/02, 37/04, 35/16, C 07 G 7/00

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
IPC 3	A 61 K 37/00, 37/02, 37/04, 35/14, 35/16, C 07 G 7/00
US Cl	424:101, 177; 260:112

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [15] with indication, where appropriate, of the relavant passages [17]	Relevant to Claim No. [19]
A	DE, A, 2 848 529 (BEHRINGWERKE AG) 29 May 1980 see inter alia the example,& EP, A, 0 011 231	
Y,A	DE, A, 2 854 381 (G A ROCK) 21 June 1979 see especially page 5, lines 17-22, and page 10, lines 8-10, & US, A, 4 203 891	
Y	DE, A, 2 916 711 (BEHRINGWERKE AG) 6 November 1980 see especially page 19	
A	US, A, 3 631 018 (BAXTER LABORATORIES, INC) 28 December 1971 see inter alia column 2, lines 64-68 and Example 1	
A	US, A, 3 920 625 (AB KABI) 18 November 1975 see column 5, lines 37-41, and column 6, lines 20-24 .../...	

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
1982-01-15	1982 -01- 2 0
International Searching Authority [1]	Signature of Authorized Officer [20]
Swedish Patent Office	Martin Hjälmdahl

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No 18
A	US, A, 4 069 216 (EDWARD SHANBROM, INC) 17 January 1978 see especially column 3, lines 57-60, & DE, A, 2 703 620	
A	US, A, 4 081 431 (BIOTEST-SERUM-INSTITUT GMBH) 28 March 1978 see Example 1	
A	US, A, 4 229 435 (B E G BLOMBÄCK & D B BESSEL) 21 October 1980 see column 1, line 67 - column 2, line 3	
A	Arkiv för kemi, Volume 12, no 36, issued 1958 (ALMQVIST & WIKSELL, Stockholm), M BLOMBÄCK, "Purification of antihemophilic globulin", see pages 387-396, especially pages 392 and 395 ("Summary")	
Y, A	British Journal of Haematology, Volume 23, no 1, issued 1972 (Oxford), C SIMONETTI et al, "Studies on the Adsorption of Factor VIII: Application to the Purification of Bovine Factor VIII", see pages 29-36, especially page 30 and page 35 ("Discussion")	